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New Opportunities in Cancer Risk Evaluation Using PCR-Based DNA Analysis for CYP2D6

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Genetic polymorphisms of drug-metabolizing enzymes, principally CYP2D6 (debrisoquine 4-hydroxylase), have long been considered influential on host responsiveness to environmental carcinogens. In several independent studies, lung cancer cases are more frequently associated with the extensive metabolizer phenotype of CYP2D6. However, assignment of phenotype has traditionally involved administration of debrisoquine and analysis of drug and metabolite concentrations in patient urine and is thus potentially confounded by concomitant drug therapy and the presence of the tumor itself. The development of molecular genotyping methods offers unique opportunities to obviate these problems and to ascertain the relationship between the presence of individual alleles and disease risk. Preliminary data are presented that indicate that the *CYP2D6* wild-type allele may be a predisposing factor in lung cancer.

Introduction

It is most appropriate that the findings in this report should be presented at the First International Conference on Environmental Mutagenesis in Human Populations at Risk in Cairo, for it was 15 years ago when an Egyptian physician, Afaf Mahgoub, working with one of us (J.R.I.) at St. Mary's Hospital Medical School in London, first discovered the genetic polymorphism in debrisoquine 4-hydroxylation (*1*). The intervening period has witnessed a renaissance in pharmacogenetics (*2*) and an explosion of new drug substrates [see Caporaso and Shaw (*3*) for a compendium] and potential roles for this polymorphic enzyme, now referred to as CYP2D6 (*4*). Between 1 and 10% of various human populations have little or no CYP2D6-mediated metabolic ability, and it was speculated over a decade ago that this might lead to differential susceptibility to lung cancer in cigarette smokers (*5*), even though no tobacco carcinogen had been identified as a CYP2D6 substrate. Only recently has it emerged that the tobacco-specific nitrosamine NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] is metabolized to mutagenic products by CYP2D6 (*6*). Various case–control studies have been carried out in lung cancer using the phenotyping test developed by Mahgoub et al. (*1*), the first of which (*7,8*) showed that the recessive poor metabolizer (PM) phenotype was reduced from 9% in controls (n = 234) to 1.4% in cases (n = 245). The lung cancer cases, all of whom were northwestern European cigarette smokers of more than 20 pack-years, matched for smoking, age, and sex with the controls, were aggregated toward the fastest metabolizing end of the spectrum. It was speculated (*7*) that these might contain a higher-than-expected proportion of homozygous dominant extensive metabolizers (EM), the apparent at-risk genotype. However, the phenotyping test is unable to distinguish homozygous from heterozygous EM subjects. Many other groups have investigated the relationship between the EM/PM phenotype and lung cancer, and, on balance, there exists a moderately strong association between the EM phenotype and lung cancer. [Readers are directed to an excellent review by Caporaso et al. (*9*)]. Now the technology has become available (*10*) to perform direct genotyping analysis for the various alleles at the *CYP2D6* gene locus that give rise to the functional polymorphism observed after the administration of debrisoquine (*1*).

Collaborating laboratories in the United States and Switzerland have principally solved much of the riddle of the debrisoquine hydroxylation polymorphism [reviewed in Gonzalez and Meyer (*11*)]. Using both restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) analyses, it has become possible to detect a number of discrete mutations that cause the deficient PM phenotype in 1–10% of populations. Figure 1 shows a simplified schematic representation of the *CYP2D* locus, which comprises one actively transcribed gene (*CYP2D6*) and two highly homologous pseudogenes (*CYP2D7P* and *CYP2D8P*). Insertions, deletions, and point mutations have all been found, and 95% of PMs can now be detected unequivocally by these methods (*10*). In addition, and for the first time, it is possible to assign correctly the hetero-

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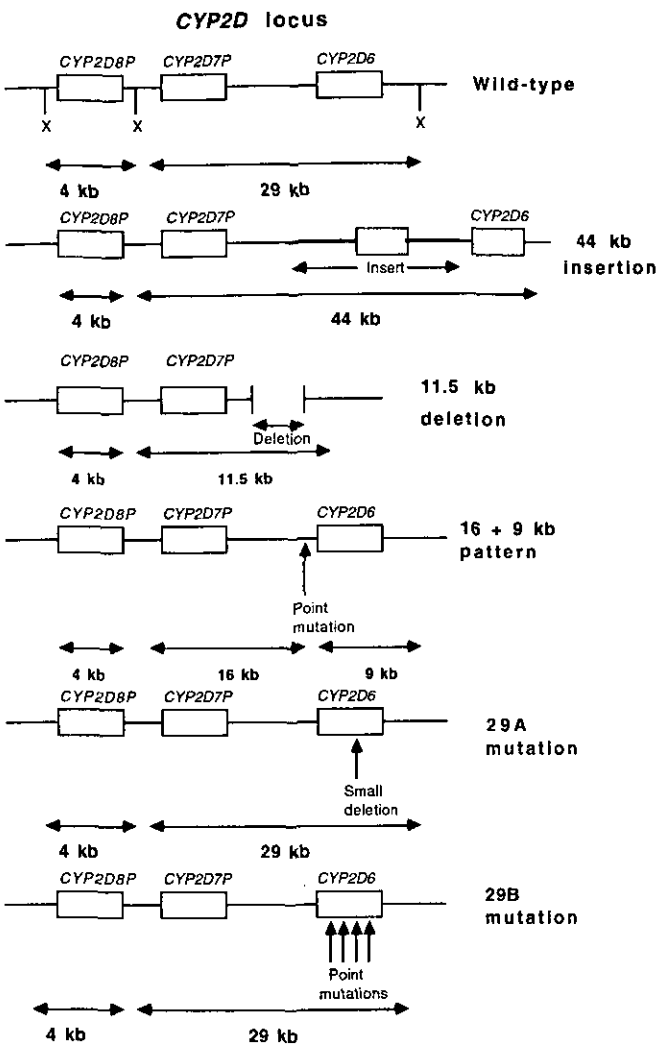

FIGURE 1. Schematic representation of the *CYP2D* locus showing the transcribed gene *CYP2D6* and the two pseudogenes *CYP2D7P* and *CYP2D8P*. Horizontal arrows represent the principal fragments after *XbaI* digestion detected on Southern blotting with a full-length cDNA probe (*10*).

zygous EM genotype in 98% of cases (*10*), thus the opportunity exists to reevaluate the lung cancer association using these methods. Although long-term studies are underway in several laboratories, we report here the rationale for such studies together with a reassessment of the original debrisoquine hydroxylation data (*7*) in lung cancer cases and controls.

Relationship between Metabolic Phenotype and *CYP2D6* Genotype

By studying a random, healthy population with both debrisoquine administration (phenotyping) and genomic DNA analysis (genotyping), together with genotyping a panel of 22 subjects previously phenotyped as PMs, we have been able to visualize the effect on debrisoquine metabolism of carrying either one (heterozygous EM) or two (PM) mutant alleles [(*10*) Fig. 2]. PMs have metabolic ratios [MR; urinary % dose and debrisoquine/% dose as 4-hydroxydebrisoquine (*1*)] greater than 10, homozygous EMs have, in almost all cases, MRs <0.6, and the heterozygotes are intermediate between the two with a modal value at 1.0. There is therefore a gene–dose effect on metabolism. For the first time this permits us to reevaluate the original data of Ayesh et al. (*7*) by estimating the proportion of cancer cases and controls who, from their MR values, were likely to have been of the homozygous dominant wild-type genotype (*wt/wt*). To accomplish this maneuver, a graph of probability of being *wt/wt* versus metabolic ratio was constructed and is shown in Figure 3. In this paper we use this relationship to estimate the number of cases and controls at each MR increment who were likely to have been *wt/wt* homozygotes, the genotype first proposed to be at highest risk of lung cancer in smokers (*7*).

Estimated Genotype Distribution in the Lung Cancer Cases and Controls of Ayesh et al.

It has been our experience that subjects phenotyped by gas chromatography at St. Mary's Hospital Medical School in the early 1980s have the same metabolic ratio values when rephenotyped in our laboratories today. We felt justified therefore in using the relationship in Figure 3 (derived from the Figure 2 data from the current study), as a calibration curve to estimate retrospectively the genotypes of the 1984 study population (*7*). This exercise yields the two distributions depicted in Figure 4. The cancer cases, as one would expect from the distribution in Figure 2, have a preponderance of *wt/wt* subjects, explaining why Ayesh et al. (*7*) observed the skewed distribution

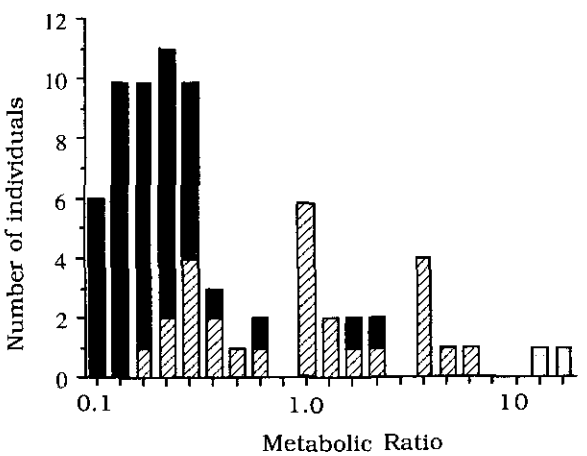

FIGURE 2. Relationship between *CYP2D6* genotype and metabolic ratio (see text). Filled bars, hatched bars, and stippled bars represent homozygous wild-type/wild-type, heterozygous wild-type/mutant, and homozygous mutant/mutant genotypes, respectively.

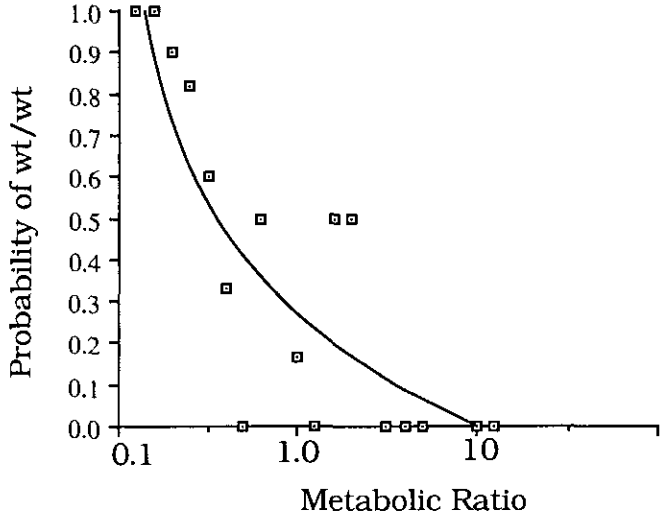

FIGURE 3. Relationship between metabolic ratio (see text) and the probability of being wild-type/wild-type genotype. Data are derived essentially from Figure 2.

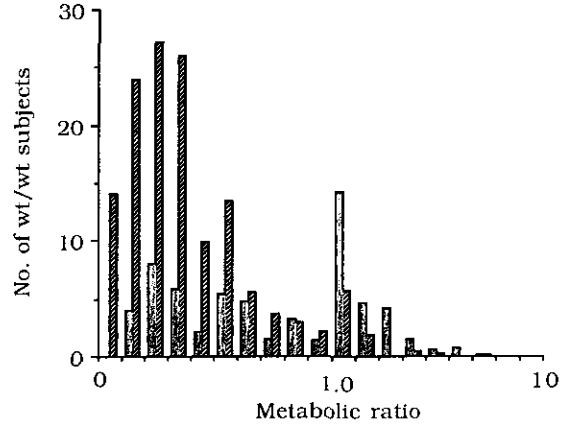

FIGURE 4. Depiction of the calculated wild-type/wild-type subjects at each incremental metabolic ratio for both lung cancer cases (▨) and controls (■) from the metabolic data of Ayesh et al. (*7*).

of cases toward a lower metabolic ratio compared to controls. The *wt/wt* genotype was estimated to occur in 56.0 and 26.7% of cases and controls, respectively, which gives an approximate relative risk (*12*) of 1.79 for the *wt/wt* genotype. In the earlier phenotypic analysis of these patients (*8*), persons with an MR value <1.0 were at 4-fold risk of lung cancer. In the analysis presented here, 35/234 (14.9%) and 127/245 (51.7%) of the controls and cases, respectively, had both MR values <1.0 and the *wt/wt* genotype. The relative risk ratio for such persons is 2.17, somewhat less than the value determined by phenotyping alone (*vide supra*). At present we are able to conclude that *wt/wt* persons are at approximately twice the risk of lung cancer than those who harbor one or two mutated alleles.

Discussion

After publication of the original study showing an association between extensive metabolism of debrisoquine and lung cancer (*7*), various groups claimed that the effect may not have been one of disease causation, but a result of the disease itself. Perhaps concomitant drug therapy, ectopic hormones, alterations in renal function, or immunological changes in the cancer group resulted in our findings. Although none of these confounders could be substantiated from the vast amount of clinical data on these patients available for epidemiological analysis, it nevertheless remained as a set of theoretical possibilities, not helped by the fact that, until 1991 (*6*), no tobacco carcinogen could be identified as a substrate of CYP2D6. The availability of recombinant DNA technology has not only permitted workers to screen promutagens and procarcinogens in human cells with enhanced cDNA-directed expression of *CYP2D6* (*6*), but also has allowed clarification of the relationship between discrete genotype and debrisoquine metabolism *in vivo*. It has indeed been confirmed that the fastest metabolizers are almost invariably of the *wt/wt* genotype. Only the ongoing and as yet unreported studies will confirm or deny the relationship between the *wt* allele and cancer risk. In the meantime, however, we have reported here an estimation of the likely number of *wt/wt* subjects in the original case–control study (*7*). These deductions lead to the expectation of finding no more than a 2-fold difference in relative risk for this *wt/wt* genotype, somewhat less than the metabolic studies would indicate.

It is probable, indeed highly likely, that several metabolic polymorphisms influence the process of chemical carcinogenesis in at-risk populations (*13*). Recognized candidates, in addition to CYP2D6, are CYP1A1 (formerly AHH) and the glutathione S-transferase GST-μ (*13*). However, data are beginning to emerge concerning polymorphisms of other candidate genes, particularly *CYP1A2* and *CYP2E1*, which also code for cytochrome P450 isozymes. Future prospects of learning considerably more about cancer risk by the study of a battery of such genes are extremely good. It is hard to envisage how functional genetic polymorphisms of these enzymes, which both activate and detoxicate environmental mutagens, would not in major part determine genotoxicity in the individual and thus cancer risk. Only by harnessing the skills of both the clinical epidemiologist and the laboratory scientist will the jigsaw finally take shape.

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